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PROVISIONAL APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53 (c).

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TITLE OF THE INVENTION (280 characters)

COMPOSITIONS AND METHODS FOR REGULATING NK CELL ACTIVITY

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☒	Specification	Number of Pages	55	☐	Applicant claims "small entity" status. ☐ "Small entity" statement attached.
☒	Drawing(s)	Number of Sheets	2	☒	Other (specify) color sheets of drawings

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Respectfully submitted,
SIGNATURE _____ DATE February 19, 2004

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☐ Additional inventors are being named on separately numbered sheets attached hereto.

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U.S. PROVISIONAL PATENT APPLICATION

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Invention: COMPOSITIONS AND METHODS FOR REGULATING NK CELL ACTIVITY

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SPECIFICATION

819025

COMPOSITIONS AND METHODS FOR REGULATING NK CELL ACTIVITY

Field of Invention

[0001] The present invention relates to antibodies, fragments and derivatives thereof that cross-react with two or more inhibitory receptors present on the cell surface of NK cells and cause a potentiation of NK cell cytotoxicity in mammalian subjects or in a biological sample. The invention also relates to methods of making such antibodies, fragments and derivatives, as well as to pharmaceutical compositions comprising the same and their uses, particularly in therapy, to increase NK cell activity or cytotoxicity in subjects.

Background

[0002] Natural killer (NK) cells are a sub-population of lymphocytes, involved in non-conventional immunity. NK cells can be obtained by various techniques known in the art, such as from blood samples, cytapheresis, collections, etc.

[0003] Characteristics and biological properties of NK cells include the expression of surface antigens including CD16, CD56 and/or CD57, and the absence of the alpha/beta or gamma/delta TCR complex expressed on the cell surface; the ability to bind to and kill cells that fail to express "self" MHC/HLA antigens by the activation of specific cytolytic enzymes; the ability to kill tumor cells or other diseased cells that express a NK activating receptor-ligand; the ability to release protein molecules called cytokines that stimulate or inhibit the immune response; and the ability to undergo multiple rounds of cell division and produce daughter cells with similar biologic properties as the parent cell. Within the context of this invention "active" NK cells designate biologically active NK cells, more particularly NK cells having the capacity of lysing target cells. For instance, an "active" NK cell is able to kill cells that express an NK activating receptor-ligand and fails to express "self" MHC/HLA antigens (KIR-incompatible cells).

[0004] Based on their biological properties, various therapeutic and vaccine strategies have been proposed in the art that rely on a modulation of NK cells. However, NK cell

activity is regulated by a complex mechanism that involves both stimulating and inhibitory signals. Accordingly, effective NK cell-mediated therapy requires both a stimulation of these cells and a neutralization of inhibitory signals.

[0005] NK cells are negatively regulated by major histocompatibility complex (MHC) class I-specific inhibitory receptors (Kärre et al., 1986; Öhlén et al, 1989). These specific receptors bind to polymorphic determinants of major histocompatibility complex (MHC) class I molecules or HLA present on other cells and inhibit natural killer (NK) cell lysis. In humans, certain members of a family of receptors termed killer Ig-like receptors (KIRs) recognize groups of HLA class I alleles.

[0006] KIRs are a large family of receptors present on certain subsets of lymphocytes, including NK cells. The nomenclature for KIRs is based upon the number of extracellular domains (KIR2D or KIR3D) and whether the cytoplasmic tail is either long (KIR2DL or KIR3DL) or short (KIR2DS or KIR3DS). Within humans, the presence or absence of a given KIR is variable from one NK cell to another within the NK population present in a single individual. Within the human population there is also a relatively high level of polymorphism of the KIR molecules with certain KIR molecules being present in some, but not all individuals. Certain KIR gene products cause stimulation of lymphocyte activity when bound to an appropriate ligand. The confirmed stimulatory KIRs all have a short cytoplasmic tail with a charged transmembrane residue that associates with an adapter molecule having an immunostimulatory motif (ITAM). Other KIR gene products are inhibitory in nature. All confirmed inhibitory KIRs have a long cytoplasmic tail and appear to interact with different subsets of HLA antigens depending upon the KIR subtype. Inhibitory KIRs display in their intracytoplasmic portion one or several inhibitory motifs that recruit phosphatases. The known inhibitory KIR receptors include members of the KIR2DL and KIR3DL subfamilies. KIR receptors having two Ig domains (KIR2D) identify HLA-C allotypes: KIR2DL2 (formerly designated p58.2) or the closely related gene product KIR2DL3 recognizes an epitope shared by group 2 HLA-C allotypes (Cw1, 3, 7, and 8), whereas KIR2DL1 (p58.1) recognizes an epitope shared by the reciprocal group 1 HLA-C allotypes (Cw2, 4, 5, and 6). The recognition by KIR2DL1 is dictated by the presence of a Lys residue at position

80 of HLA-C alleles. KIR2DL2 and KIR2DL3 recognition is dictated by the presence of an Asn residue at position 80. Importantly the great majority of HLA-C alleles have either an Asn or a Lys residue at position 80. One KIR with three Ig domains, KIR3DL1 (p70), recognizes an epitope shared by HLA-Bw4 alleles. Finally, a homodimer of molecules with three Ig domains KIR3DL2 (p140) recognizes HLA-A3 and -A11.

[0007] Although inhibitory KIRs and other class-I inhibitory receptors (Moretta et al, 1997; Valiante et al, 1997a; Lanier, 1998) may be co-expressed by NK cells, in any given individual's NK repertoire there are cells that express a single KIR and thus, the corresponding NK cells are blocked only by cells expressing a specific class I allele group.

[0008] NK cell population or clones that are KIR mismatched, i.e., population of NK cells that express KIR that are not compatible with a HLA molecules of a host, have been shown to be the most likely mediators of the graft anti leukemia effect seen in allogeneic transplantation (Ruggeri et al., 2002). One way of reproducing this effect in a given individual would be to use reagents that block the KIR/HLA interaction.

[0009] Monoclonal antibodies specific for KIR2DL1 have been shown to block the interaction of KIR2DL1 with Cw4 (or the like) alleles (Moretta et al., 1993). Monoclonal antibodies against KIR2DL2/3 have also been described that block the interaction of KIR2DL2/3 with HLACw3 (or the like) alleles (Moretta et al., 1993). However, the use of such reagents in clinical situations would require the development of two therapeutic mAbs to treat all patients, regardless of whether any given patient was expressing class 1 or class 2 HLA-C alleles. Moreover, one would have to pre-determine which HLA type each patient was expressing before deciding which therapeutic antibody to use, thus resulting in much higher cost of treatment.

[0010] Watzl et al., Tissue Antigens, 56, p. 240 (2000) produced cross-reacting antibodies recognizing multiple isotypes of KIRs, but those antibodies did not exhibit potentiation of NK cell activity. G. M. Spaggiara et al., Blood, 100, pp. 4098-4107 (2002) carried out experiments utilizing numerous monoclonal antibodies against

various KIRs. One of those antibodies, NKVSF1, was said to recognize a common epitope of CD158a KIR2DL1), CD158b (KIR2DL2) and p50.3 (KIR2DS4). It is not suggested that NKVSF1 can potentiate NK cell activity and there is no suggestion that it could be used as a therapeutic. Accordingly, practical and effective approaches in the modulation of NK cell activity have not been made available so far in the art and still require HLA allele-specific intervention using specific reagents.

Summary of the Invention

[0011] The present invention now provides novel antibodies, compositions and methods that overcome the current difficulty in NK cell activation. More particularly, the present invention provides a single antibody that facilitates the activation of human NK cells in virtually all individuals. The invention discloses novel specific antibodies that cross react with various inhibitory KIR groups and neutralize their inhibitory signals, resulting in potentiation of NK cell cytotoxicity in NK cells expressing such inhibitory KIR receptors. This ability to cross-react with multiple KIR gene products allows these reagents to be effectively used in most human subjects to increase NK cell activity, without the burden or expense of pre-determining the HLA type of the subject.

[0012] In a first aspect, the invention provides an antibody, as well as fragments and derivatives thereof, wherein said antibody, fragment or derivative cross reacts with at least two inhibitory KIR receptors at the surface of NK cells, neutralizes their inhibitory signals and potentiates the activity of those cells. More preferably, the antibody binds a common determinant of human KIR2DL receptors. Even more specifically, the antibody of this invention binds at least KIR2DL1, KIR2DL2 and KIR2DL3 receptors. For the purposes of this invention, the term "KIR2DL2/3" refers to either or both of the KIR2DL2 and KIR2DL3 receptors. These two receptors have a very high homology, are presumably allelic forms of the same gene, and are considered by the art to be interchangeable. Accordingly, KIR2DL2/3 is considered to be a single inhibitory KIR molecule for the purposes of this invention and therefore an antibody that cross-reacts with only KIR2DL2 and KIR2DL3 and no other inhibitory KIR receptors is not within the scope of this invention.

[0013] The antibody of this invention specifically inhibits binding of HLA molecules to at least two inhibitory KIR receptors and facilitates NK cell activity. Both activities are inferred by the term "neutralize the inhibitory activity of KIR," as used herein. The ability of the antibodies of this invention to "facilitate NK cell activity," "facilitate NK cell cytotoxicity," "facilitate NK cells," "potentiate NK cell activity, " "potentiate NK cell cytotoxicity," or "potentiate NK cells" in the context of this invention means that the antibody causes NK cells expressing an inhibitory KIR receptor on their surface to be capable of lysing cells that express on their surface a corresponding ligand (HLA antigen) for that particular inhibitory KIR receptor. Preferably, the antibody of this invention specifically inhibits the binding of HLA-c molecules to KIR2DL1 and KIR2DL2/3 receptors. More preferably, the antibody facilitates NK cell activity in vivo.

[0014] Because at least one of KIR2DL1 or KID2DL2/3 is present in at least 90% of the human population, the more preferred antibodies of this invention are sufficient for covering most of the HLA-C allotypes, respectively group 1 HLA-C allotypes and group 2 HLA-C allotypes. Thus, the compositions of this invention may be used to effectively activate or potentiate NK cells in most human individuals, typically in about 90% of human individuals or more. Accordingly, a single antibody composition may be used to treat most human subjects, and there is seldom need to determine allelic groups or to use antibody cocktails.

[0015] The invention demonstrates, for the first time, that cross-reactive and neutralizing antibodies against inhibitory KIRs may be generated, and that such antibodies allow effective activation of NK cells in a broad range of human groups.

[0016] A particular object of this invention thus resides in an antibody, wherein said antibody binds a common determinant present in both KIR2DL1 and KIR2DL2/3 human receptors and reverses inhibition of NK cell cytotoxicity mediated by these KIRs. The antibody more specifically binds to substantially the same epitope as monoclonal antibody DF200 produced by hybridoma DF200. The terms "binds to about, substantially or essentially the same, or the same, epitope as" the monoclonal antibody

DF200 mean that an antibody competes in a binding assay using either recombinant KIR molecules or surface expressed KIR molecules, with the monoclonal antibody DF200. An antibody that "competes with DF200" is one that recognizes, binds to or has immunospecificity for substantially or essentially the same, or the same, epitope or "epitopic site" as the monoclonal antibody DF200. Thus, such a competing antibody is able to effectively compete with DF200 for binding to a KIR receptor at the surface of a human NK cell or compete for binding on recombinant KIR molecules.

[0017] In a preferred embodiment, the antibody of this invention is a monoclonal antibody. The most preferred antibody of this invention is monoclonal antibody DF200 produced by hybridoma DF200.

[0018] The invention also encompasses fragments and derivatives of the antibodies described having substantially the same antigen specificity and activity (e.g., which can cross-react with the parent antibody and which potentiate the cytotoxic activity of NK cells expressing inhibitory KIR receptors), including, without limitation, a Fab fragment, a Fab'2 fragment, a CDR and a ScFv. Furthermore, the antibodies of this invention may be humanized, human, or chimeric.

[0019] The invention also discloses methods of producing an antibody other than NKVSF1 which cross-reacts with multiple KIR2DL gene products and which neutralizes the inhibitory activity of such KIRs, said method comprising the steps of:

(a) immunizing a non-human mammal with an immunogen comprising a KIR2DL polypeptide;

(b) preparing antibodies from said immunized animal, wherein said antibodies bind said KIR2DL polypeptide,

(c) selecting antibodies of (b) that cross react with at least two different KIR2DL gene products, and

(d) selecting antibodies of (c) that potentiate NK cells.

[0020] The invention also relates to pharmaceutical compositions comprising an antibody as disclosed above or a fragment or derivative thereof.

[0021] The invention also provides methods of regulating human NK cell activity in vitro, ex vivo or in vivo, comprising contacting human NK cells with an antibody or a fragment or derivative as defined above or a pharmaceutical composition as described above. Most preferred methods use the pharmaceutical compositions of this invention and are directed at increasing the cytotoxic activity of human NK cells, most preferably ex vivo or in vivo, in a subject having a cancer, infectious or immune disease.

Brief Description of the Drawings

[0022] Figure 1 depicts monoclonal antibody DF200 binding to a common determinant of various human KIR2DL receptors.

[0023] Figure 2 depicts monoclonal antibody DF200 neutralizing the KIR2DL-mediated inhibition of KIR2DL1 positive NK cell cytotoxicity on Cw4 positive target cells.

Detailed Description of the Invention

Antibodies

[0024] The present invention provides novel antibodies and fragments or derivatives thereof that bind common determinants of human inhibitory KIR receptors, preferably a determinant present on at least two different KIR2DL gene products, and cause potentiation of NK cells expressing at least one of those KIR receptors. The invention discloses, for the first time, that such cross-reacting and neutralizing antibodies can be produced, which represents an unexpected result and opens an avenue towards novel and effective NK-based therapies, particularly in human subjects. In a preferred embodiment, the antibody is not monoclonal antibody NKVSF1.

[0025] Within the context of this invention a "common determinant" designates a determinant or epitope that is shared by several gene products of the human inhibitory KIR receptors. Preferably, the common determinant is shared by at least two members of the KIR2DL receptor group. More preferably, the determinant is shared by at least

KIR2DL1 and KIR2DL2/3. Certain antibodies of this invention may, in addition to recognizing multiple gene products of KIR2DL, also recognize determinants present on other inhibitory KIRs, such as gene product of the KIR3DL receptor group. The determinant or epitope may represent a peptide fragment or a conformational epitope shared by said members. In a more specific embodiment, the antibody of this invention specifically binds to substantially the same epitope recognized by monoclonal antibody DF200. This determinant is present on both KIR2DL1 and KIR2DL2/3.

[0026] Within the context of this invention, the term antibody that "binds" a common determinant designates an antibody that binds said determinant with specificity and/or affinity

[0027] The term "antibody," as used herein, refers to polyclonal and monoclonal antibodies, as well as to fragments and derivatives of said polyclonal and monoclonal antibodies. Depending on the type of constant domain in the heavy chains, antibodies are assigned to one of five major classes: IgA, IgD, IgE, IgG, and IgM. Several of these are further divided into subclasses or isotypes, such as IgG1, IgG2, IgG3, IgG4, and the like. The heavy-chain constant domains that correspond to the difference classes of immunoglobulins are termed "alpha," "delta," "epsilon," "gamma" and "mu," respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known. IgG and/or IgM are the preferred classes of antibodies employed in this invention because they are the most common antibodies in the physiological situation and because they are most easily made in a laboratory setting. Preferably the antibody of this invention is a monoclonal antibody. Because one of the goals of the invention is to block the interaction of an inhibitory KIR and its corresponding HLA ligand in vivo without depleting the NK cells, isotypes corresponding to Fc receptors that mediate low effector function, such as IgG4, are preferred.

[0028] The antibodies of this invention may be produced by a variety of techniques known in the art. Typically, they are produced by immunization of a non-human animal, preferably a mouse, with an immunogen comprising an inhibitory KIR polypeptide,

preferably a KIR2DL polypeptide, more preferably a human KIR2DL polypeptide. The inhibitory KIR polypeptide may comprise the full length sequence of a human inhibitory KIR polypeptide, or a fragment or derivative thereof, typically an immunogenic fragment, i.e., a portion of the polypeptide comprising an epitope exposed on the surface of the cell expressing an inhibitory KIR receptor. Such fragments typically contain at least 7 consecutive amino acids of the mature polypeptide sequence, even more preferably at least 10 consecutive amino acids thereof. They are essentially derived from the extra-cellular domain of the receptor. Even more preferred is a human KIR2DL polypeptide which includes at least one, more preferably both, extracellular Ig domains, of the full length KIRDL polypeptide and is capable of mimicking at least one conformational epitope present in a KIR2DL receptor. In other embodiments, said polypeptide comprises at least 8 consecutive amino acids of an extracellular Ig domain of amino acid positions 1-224 of the KIR2DL1 polypeptide (amino acid numbering of according to PROW web site describing the KIR gene family, http://www.ncbi.nlm.nih.gov/prow/guide/1326018082.htm)

[0029] In a most preferred embodiment, the immunogen comprises a wild-type human KIR2DL polypeptide in a lipid membrane, typically at the surface of a cell. In a specific embodiment, the immunogen comprises intact NK cells, particularly intact human NK cells, optionally treated or lysed.

[0030] The step of immunizing a non-human mammal with an antigen may be carried out in any manner well known in the art for stimulating the production of antibodies in a mouse (see, for example, E. Harlow and D. Lane, Antibodies: A Laboratory Manual., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1988)). The immunogen is then suspended or dissolved in a buffer, optionally with an adjuvant, such as complete Freund's adjuvant. Methods for determining the amount of immunogen, types of buffers and amounts of adjuvant are well known to those of skill in the art and are not limiting in any way on the present invention. These parameters may be different for different immunogens, but are easily elucidated.

[0031] Similarly, the location and frequency of immunization sufficient to stimulate the

production of antibodies is also well known in the art. In a typical immunization protocol, the non-human animals are injected intraperitoneally with antigen on day 1 and again about a week later. This is followed by recall injections of the antigen around day 20, optionally with adjuvant such as incomplete Freund's adjuvant. The recall injections are performed intravenously and may be repeated for several consecutive days. This is followed by a booster injection at day 40, either intravenously or intraperitoneally, typically without adjuvant. This protocol results in the production of antigen-specific antibody-producing B cells after about 40 days. Other protocols may also be utilized as long as they result in the production of B cells expressing an antibody directed to the antigen used in immunization.

[0032] For polyclonal antibody preparation, serum is obtained from an immunized non-human animal and the antibodies present therein isolated by well-known techniques. The serum may be affinity purified using any of the immunogens set forth above linked to a solid support so as to obtain antibodies that react with inhibitory KIR receptors.

[0033] In an alternate embodiment, lymphocytes from an unimmunized non-human mammal are isolated, grown in vitro, and then exposed to the immunogen in cell culture. The lymphocytes are then harvested and the fusion step described below is carried out.

[0034] For monoclonal antibodies, the next step is the isolation of splenocytes from the immunized non-human mammal and the subsequent fusion of those splenocytes with an immortalized cell in order to form an antibody-producing hybridoma. The isolation of splenocytes from a non-human mammal is well-known in the art and typically involves removing the spleen from an anesthetized non-human mammal, cutting it into small pieces and squeezing the splenocytes from the splenic capsule and through a nylon mesh of a cell strainer into an appropriate buffer so as to produce a single cell suspension. The cells are washed, centrifuged and resuspended in a buffer that lyses any red blood cells. The solution is again centrifuged and remaining lymphocytes in the pellet are finally resuspended in fresh buffer.

[0035] Once isolated and present in single cell suspension, the lymphocytes are fused to an immortal cell line. This is typically a mouse myeloma cell line, although many other

immortal cell lines useful for creating hybridomas are known in the art. Preferred murine myeloma lines include, but are not limited to, those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, Calif. U.S.A., X63 Ag8653 and SP-2 cells available from the American Type Culture Collection, Rockville, Maryland U.S.A. The fusion is effected using polyethylene glycol or the like. The resulting hybridomas are then grown in selective media that contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

[0036] The hybridomas are typically grown on a feeder layer of macrophages. The macrophages are preferably from littermates of the non-human mammal used to isolate splenocytes and are typically primed with incomplete Freund's adjuvant or the like several days before plating the hybridomas. Fusion methods are described in Goding, "Monoclonal Antibodies: Principles and Practice," pp. 59-103 (Academic Press, 1986), the disclosure of which is herein incorporated by reference.

[0037] The cells are allowed to grow in the selection media for sufficient time for colony formation and antibody production. This is usually between 7 and 14 days. The hybridoma colonies are then assayed for the production of antibodies that cross-react with multiple inhibitory KIR receptor gene products. The assay is typically a colorimetric ELISA-type assay, although any assay may be employed that can be adapted to the wells that the hybridomas are grown in. Other assays include immunoprecipitation and radioimmunoassay. The wells positive for the desired antibody production are examined to determine if one or more distinct colonies are present. If more than one colony is present, the cells may be re-cloned and grown to ensure that only a single cell has given rise to the colony producing the desired antibody. Positive wells with a single apparent colony are typically recloned and re-assayed to insure only one monoclonal antibody is being detected and produced.

[0038] Antibodies may also be produced by selection of combinatorial libraries of immunoglobulins, as disclosed for instance in Ward et al., Nature, 341 (1989) p. 544).

[0039] The antibodies of this invention are able to neutralize the KIR-mediated inhibition of NK cell cytotoxicity; particularly inhibition mediated by KIR2DL receptors and more particularly at least both the KIR2DL1 and KIR2DL2/3 inhibition. These antibodies are thus "neutralizing" or "inhibitory" antibodies, in the sense that they block, at least partially, the inhibitory signaling pathway mediated by KIR receptors. More importantly, this inhibitory activity is displayed with respect to several types of inhibitory KIR receptors, preferably several KIR2DL receptor gene products, and more preferably at least both KIR2DL1 and KIR2DL2/3 so that these antibodies may be used in various subjects with high efficacy. Inhibition of KIR-mediated inhibition of NK cell cytotoxicity can be assessed by various assays or tests, such as binding or cellular assays.

[0040] Once an antibody that cross reacts with multiple inhibitor KIR receptors is identified, it is tested for its ability to neutralize the inhibitory effect of those KIR receptors in intact NK cells. In a specific variant, the neutralizing activity is illustrated by the capacity of said antibody to reconstitute lysis by KIR2DL-positive NK clones of HLA-C positive targets. In another specific embodiment, the neutralizing activity of the antibody is defined by the ability of the antibody to inhibit the binding of HLA-C molecules to KIR2DL1 and KIR2DL3 (or the closely related KIR2DL2) receptors, further preferably as it is the capacity of the antibody to alter:

- the binding of a HLA-c molecule selected from Cw1, Cw3, Cw7, and Cw8 (or of a HLA-C molecule having an Asn residue at position 80) to KIR2DL2/3; and
- the binding of a HLA-C molecule selected from Cw2, Cw4, Cw5 and Cw6 (or of a HLA-C molecule having a Lys residue at position 80) to KIR2DL1.

[0041] In another variant, the inhibitory activity of an antibody of this invention can be assessed in a cell based cytotoxicity assay, as disclosed in the examples.

[0042] In another variant, the inhibitory activity of an antibody of this invention can be assessed in a cytokine-release assay, wherein NK cells are incubated with the test antibody to stimulate NK cell cytokine production (for example IFN-γ and GM-CSF production). In an exemplary protocol, IFN-γ production from PBMC is assessed by cell surface and intracytoplasmic staining and analysis by flow cytometry after 4 days in culture. Briefly, Brefeldin A (Sigma Aldrich) is added at a final concentration of 5 μg/ml for the last 4 hours of culture. The cells are then incubated with anti-CD3 and anti-CD56 mAb prior to permeabilization (IntraPrep™; Beckman Coulter) and staining with PE-anti-IFN-γ or PE-IgG1 (Pharmingen). GM-CSF and IFN-γ production from polyclonal activated NK cells are measured in supernatants using ELISA (GM-CSF: DuoSet Elisa, R&D Systems, Minneapolis, MN; IFN-γ: OptE1A set, Pharmingen).

[0043] The antibodies of this invention may partially or fully neutralize the KIR-mediated inhibition of NK cell cytotoxicity. The term "neutralize KIR-mediated inhibition of NK cell cytotoxicity," as used herein means the ability to increase at least 20%, preferably at least 30%, 40% or 50% or more of specific lysis as measured by a classical chromium release test of cytotoxicity, compared with the level of specific lysis obtained without antibody when an NK cell population expressing a given KIR is put in contact with a target cell expressing the cognate MHC class I molecule (recognized by the KIR expressed on NK cell). For example, preferred antibodies of this invention are able to induce the lysis of matched or HLA compatible or autologous target cell populations, i.e., cell populations that would not be effectively lysed by NK cells in the absence of said antibody. Accordingly, the antibodies of this invention may also be defined as facilitating NK cell activity in vivo.

[0044] Alternatively, the term "neutralize KIR mediated inhibition" means that in a chromium assay using an NK cell clone expressing one or several inhibitory KIRs and a target cell expressing only one HLA allele that is recognized by one of the KIRs on the NK clone, the level of cytotoxicity obtained with the antibody should be at least 60 % preferably 70 % or more of the cytotoxicity obtained with a known blocking anti MHC class I molecule, such as W6/32 anti MHC class I antibody.

[0045] In a specific embodiment, the antibody binds substantially the same epitope as monoclonal antibody DF200 (produced by hybridoma DF200). Such antibodies are referred to herein as "DF-200 like antibodies." In a further preferred embodiment, the antibody is a monoclonal antibody. More preferred "DF-200 like antibodies" of this invention are antibodies other than the monoclonal antibody NKVSF1. Most preferred is monoclonal antibody DF200 (produced by hybridoma DF200).

[0046] The term "binds to substantially the same epitope or determinant as" the monoclonal antibody DF200 means that an antibody "competes" with DF200. The identification of one or more antibodies that bind(s) to substantially the same epitope as the monoclonal antibody DF200 can be readily determined using any one of variety of immunological screening assays in which antibody competition can be assessed. All such assays are routine in the art (see, e.g., U.S. Pat. No. 5,660,827, issued Aug. 26, 1997, which is specifically incorporated herein by reference). It will be understood that actually determining the epitope to which DF200 binds is not in any way required to identify an antibody that binds to the same or substantially the same epitope as the monoclonal antibody DF200.

[0047] For example, where the test antibodies to be examined are obtained from different source animals, or are even of a different Ig isotype, a simple competition assay may be employed in which the control (DF200) and test antibodies are admixed (or pre-adsorbed) and applied to a sample containing both KIR2DL1 and KIR2DL2/3, each of which is known to be bound by DF-200. Protocols based upon ELISAs, radioimmunoassays, Western blotting and the use of BIACORE (as set forth in the example section) are suitable for use in such simple competition studies.

[0048] In certain embodiments, one would pre-mix the control antibodies (DF200) with varying amounts of the test antibodies (e.g., 1:10 or 1:100) for a period of time prior to applying to the inhibitory KIR antigen sample. In other embodiments, the control and varying amounts of test antibodies can simply be admixed during exposure to the KIR antigen sample. As long as one can distinguish bound from free antibodies (e.g., by

using separation or washing techniques to eliminate unbound antibodies) and DF200 from the test antibodies (e.g., by using species- or isotype-specific secondary antibodies or by specifically labeling DF200 with a detectable label) one will be able to determine if the test antibodies reduce the binding of DF200 to the two different KIR2DL antigens, indicating that the test antibody recognizes substantially the same epitope as DF200. The binding of the (labeled) control antibodies in the absence of a completely irrelevant antibody would be the control high value. The control low value would be obtained by incubating the labeled (DF200) antibodies with unlabelled antibodies of exactly the same type (DF200), where competition would occur and reduce binding of the labeled antibodies. In a test assay, a significant reduction in labeled antibody reactivity in the presence of a test antibody is indicative of a test antibody that recognizes substantially the same epitope, i.e., one that "cross-reacts" with the labeled (DF200) antibody. Any test antibody that reduces the binding of DF200 to each of KIR2DL1 and KIR2DL2/3 antigens by at least 50% or more preferably 70%, at any ratio of DF200:test antibody between about 1:10 and about 1:100 is considered to be an antibody that binds to substantially the same epitope or determinant as DF200. Preferably, such test antibody will reduce the binding of DF200 to each of the KIR2DL antigens by at least 90%.

[0049] For example, competition can be assessed by a flow cytometry test. Cells bearing a given KIR are incubated first with DF200 and then with the test antibody labeled with a fluorochrome or biotin. The antibody is said to compete with DF200 if the binding obtained with preincubation with saturating amount of DF200 is 80%, preferably, 50%, 40% or less than the binding (as measured by mean of fluorescence) obtained by the antibody without preincubation with DF200. Alternatively, an antibody is said to compete with DF200 if the binding obtained with a labeled DF200 (by a fluorochrome or biotin) on cells preincubated with saturating amount of test antibody is 80%, preferably 50%, 40%, or less than the binding obtained without preincubation with the antibody.

[0050] In one preferred example, a simple competition assay may be employed in which a the test antibody is pre-adsorbed and applied at saturating concentration to a surface onto which is immobilized both KIR2DL1 and KIR2DL2/3, each of which is known to

be bound by DF-200. The surface is preferably a BIACORE chip. The control antibody (DF200) is then brought into contact with the surface at KIR2DL1 and KIR2DL2/3-saturating concentration and the KIR2DL1 and KIR2DL2/3 surface binding of the control antibody is measured. This binding of the control antibody is compared with the binding of the control antibody to the KIR2DL1 and KIR2DL2/3-containing surface in the absence of test antibody. In a test assay, a significant reduction in binding of the KIR2DL1 and KIR2DL2/3-containing surface by the control antibody in the presence of a test antibody is indicative of a test antibody that recognizes substantially the same epitope, i.e., one that "cross-reacts" with the control antibody. Any test antibody that reduces the binding of control (such as DF200) antibody to each of KIR2DL1 and KIR2DL2/3 antigens by at least 30% or more preferably 40% is considered to be an antibody that binds to substantially the same epitope or determinant as DF200. Preferably, such test antibody will reduce the binding of DF200 to each of the KIR2DL antigens by at least 50%. It will be appreciated that the order of control and test antibodies can be reversed, that is the control antibody is first bound to the surface and the test antibody is brought into contact with the surface thereafter. Preferably, the antibody having higher affinity for KIR2DL1 and KIR2DL2/3 antigens is bound to the KIR2DL1 and KIR2DL2/3-containing surface first since it will be expected that the decrease in binding seen for the second antibody (assuming the antibodies are cross-reacting) will be of greater magnitude. Further examples of such assays are provided in the Examples and in Saunal and Regenmortel, (1995) J. Immunol. Methods 183: 33-41, the disclosure of which is incorporated herein by reference.

[0051] Upon immunization and production of monoclonal antibodies, particular selection steps may be performed to isolate antibodies as claimed. In this regard, in a specific embodiment, the invention also relates to methods of producing such antibodies, comprising:

(a) immunizing a non-human mammal with an immunogen comprising an inhibitory KIR polypeptide;

(b) preparing antibodies from said immunized animal, wherein said antibodies bind said KIR polypeptide,

(c) selecting antibodies of (b) that cross react with at least two different inhibitory KIR gene products, and

(d) selecting antibodies of (c) that are capable of neutralizing KIR-mediated inhibition of NK cell cytotoxicity on a population of NK cells expressing said at least two different human inhibitory KIR receptor gene products.

The selection of an antibody that cross-reacts with at least two different inhibitory KIR gene products may be achieved by screening the antibody against two or more different inhibitory KIR antigens, as described above.

[0052] In a more preferred embodiment, the antibodies prepared in step (b) are monoclonal antibodies. Thus, the term "preparing antibodies from said immunized animal," as used herein, includes obtaining B-cells from an immunized animal and using those B cells to produce a hybridoma that expresses antibodies, as well as obtaining antibodies directly from the serum of an immunized animal. In another preferred embodiment, the antibodies selected in step (c) are those that cross-react with at least KIR2DL1 and KIR2DL2/3.

[0053] In yet another preferred embodiment, the antibodies selected in step (d) cause at least a 10 % augmentation in NK cytotoxicity mediated by NK cells displaying at least one KIR recognized by the antibody, preferably at least a 40 or 50% augmentation in NK cytotoxicity, or more preferably at least a 70% augmentation in NK cytotoxicity, as measured in a standard chromium release assay towards a target cell expressing cognate HLA class I molecule. Alternatively, the antibodies selected in step (d) when used in a chromium assay employing an NK cell clone expressing one or several inhibitory KIRs and a target cell expressing only one HLA allele that is recognized by one of the KIRs on the NK clone, the level of cytotoxicity obtained with the antibody should be at least 60 % preferably 70 % or more of the cytotoxicity obtained with a blocking anti MHC class I mAb such as W6/32 anti MHC class I antibody.

[0054] The order of steps (c) and (d) can be changed. Optionally, the method may further comprise additional steps of making fragments or derivatives of the monoclonal antibody, as disclosed below. In preferred embodiment, the non-human animal is a

mammal, such as a rodent (e.g., mouse, rat, etc.), bovine, porcine, horse, rabbit, goat, sheep, etc. Also, the non-human mammal may be genetically modified or engineered to produce "human" antibodies, such as the Xenomouse™ (Abgenix) or HuMAb-Mouse™ (Medarex).

[0055] In another variant, the method comprises:

(a) selecting, from a library or repertoire, a monoclonal antibody or a fragment or derivative thereof that cross reacts with at least two different human inhibitory KIR2DL receptor gene products, and

(b) selecting an antibody of (a) that is capable of neutralizing KIR-mediated inhibition of NK cell cytotoxicity on a population of NK cells expressing said at least two different human inhibitory KIR2DL receptor gene products.

[0056] The repertoire may be any (recombinant) repertoire of antibodies or fragments thereof, optionally displayed by any suitable structure (e.g., phage, bacteria, synthetic complex, etc.). Selection of inhibitory antibodies may be performed as disclosed above and further illustrated in the examples.

[0057] According to another embodiment, the invention provides a hybridoma comprising a B cell from a non-human host, wherein said B cell produces an antibody that binds a determinant present on at least two different human inhibitory KIR receptor gene products and said antibody is capable of neutralizing the inhibitory activity of said receptors. More preferably, the hybridoma of this invention is not a hybridoma that produces the monoclonal antibody NKVSF1. The hybridoma according to this invention is created as described above by the fusion of splenocytes from the immunized non-human mammal with an immortal cell line. Hybridomas produced by this fusion are screened for the presence of such a cross-reacting antibody as previously set forth. Preferably, the hybridoma produces an antibody the recognizes a determinant present on at least two different KIR2DL gene products, and cause potentiation of NK cells expressing at least one of those KIR receptors. Even more preferably, the hybridoma produces an antibody that binds to substantially the same epitope or determinant as DF200 and which potentiates NK cell activity. Most preferably, that hybridoma is

hybridoma DF200 which produces monoclonal antibody DF200.

[0058] Hybridomas that are confirmed to be producing a monoclonal antibody of this invention are then grown up in larger amounts in an appropriate medium, such as DMEM or RPMI-1640. Alternatively, the hybridoma cells can be grown in vivo as ascites tumors in an animal.

[0059] After sufficient growth to produce the desired monoclonal antibody, the growth media containing monoclonal antibody (or the ascites fluid) is separated away from the cells and the monoclonal antibody present therein is purified. Purification is typically achieved by gel electrophoresis, dialysis, chromatography using protein A or protein G-Sepharose, or an anti-mouse Ig linked to a solid support such as agarose or Sepharose beads (all described, for example, in the Antibody Purification Handbook, Amersham Biosciences, publication No. 18-1037-46, Edition AC, the disclosure of which is hereby incorporated by reference). The bound antibody is typically eluted from protein A/protein G columns by using low pH buffers (glycine or acetate buffers of pH 3.0 or less) with immediate neutralization of antibody-containing fractions. These fractions are pooled, dialyzed, and concentrated as needed.

[0060] According to an alternate embodiment, the DNA encoding an antibody that binds a determinant present on at least two different human inhibitory KIR receptor gene products, is isolated from the hybridoma of this invention, placed in an appropriate expression vector for transfection into an appropriate host. The host is then used for the recombinant production of the antibody, or variants thereof, such as a humanized version of that monoclonal antibody, active fragments of the antibody or chimeric antibodies comprising the antigen recognition portion of the antibody. Preferably, the DNA used in this embodiment encodes an antibody that recognizes a determinant present on at least two different KIR2DL gene products, and cause potentiation of NK cells expressing at least one of those KIR receptors. Even more preferably, the DNA encodes an antibody that binds to substantially the same epitope or determinant as DF200 and which potentiates NK cell activity. Most preferably, that DNA encodes monoclonal antibody DF200.

[0061] DNA encoding the monoclonal antibodies of the invention is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). Once isolated, the DNA can be placed into expression vectors, which are then transfected into host cells such as E. coli cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Recombinant expression in bacteria of DNA encoding the antibody is well known in the art (see, for example, Skerra et al., Curr. Opinion in Immunol., 5, pp. 256 (1993); and Pluckthun, Immunol. Revs., 130, pp. 151 (1992).

Fragments and Derivatives of a Monoclonal Antibody

[0062] Fragments and derivatives of antibodies of this invention (which are encompassed by the term "antibody" or "antibodies" as used in this application), preferably a DF-200-like antibody, can be produced by techniques that are known in the art. "Immunoreactive fragments" comprise a portion of the intact antibody, generally the antigen binding site or variable region. Examples of antibody fragments include Fab, Fab', Fab'-SH, $F(ab')_2$, and Fv fragments; diabodies; any antibody fragment that is a polypeptide having a primary structure consisting of one uninterrupted sequence of contiguous amino acid residues (referred to herein as a "single-chain antibody fragment" or "single chain polypeptide"), including without limitation (1) single-chain Fv (scFv) molecules (2) single chain polypeptides containing only one light chain variable domain, or a fragment thereof that contains the three CDRs of the light chain variable domain, without an associated heavy chain moiety and (3) single chain polypeptides containing only one heavy chain variable region, or a fragment thereof containing the three CDRs of the heavy chain variable region, without an associated light chain moiety; and multispecific antibodies formed from antibody fragments. For instance, Fab or F(ab')2 fragments may be produced by protease digestion of the isolated antibodies, according to conventional techniques. It will be appreciated that immunoreactive fragments can be modified using known methods, for example to slow clearance in vivo and obtain a more

desirable pharmacokinetic profile the fragment may be modified with polyethylene glycol (PEG). Method for coupling and site-specifically conjugating PEG to a Fab' fragment are described in Leong et al, Cytokine 16(3):106-119 (2001) and Delgado et al, Br. J. Cancer 73(2):175-182 (1996), the disclosures of which are incorporated herein by reference.

[0063] Alternatively, the DNA of a hybridoma producing an antibody of this invention, preferably a DF-200-like antibody, may be modified so as to encode for a fragment of this invention. The modified DNA is then inserted into an expression vector and used to transform or transfect an appropriate cell, which then expresses the desired fragment.

[0064] In an alternate embodiment, the DNA of a hybridoma producing an antibody of this invention, preferably a DF-200-like antibody, can be modified prior to insertion into an expression vector, for example, by substituting the coding sequence for human heavy- and light-chain constant domains in place of the homologous non-human sequences (e.g., Morrison et al., Proc. Natl. Acad. Sci. U.S.A., 81, pp. 6851 (1984)), or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. In that manner, "chimeric" or "hybrid" antibodies are prepared that have the binding specificity of the original antibody. Typically, such non-immunoglobulin polypeptides are substituted for the constant domains of an antibody of the invention.

[0065] Thus, according to another embodiment, the antibody of this invention, preferably a DF-200-like antibody, is humanized. "Humanized" forms of antibodies according to this invention are specific chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$, or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from the murine immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementary-determining region (CDR) of the recipient are replaced by residues from a CDR of the original antibody (donor antibody) while maintaining the desired specificity, affinity, and capacity of the original antibody. In some instances, Fv framework residues of the human immunoglobulin may

be replaced by corresponding non-human residues. Furthermore, humanized antibodies can comprise residues that are not found in either the recipient antibody or in the imported CDR or framework sequences. These modifications are made to further refine and optimize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of the original antibody and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details see Jones et al., Nature, 321, pp. 522 (1986); Reichmann et al., Nature, 332, pp. 323 (1988); and Presta, Curr. Op. Struct. Biol., 2, pp. 593 (1992).

[0066] Methods for humanizing the antibodies of this invention are well known in the art. Generally, a humanized antibody according to the present invention has one or more amino acid residues introduced into it from the original antibody. These murine or other non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones et al., Nature, 321, pp. 522 (1986); Riechmann et al., Nature, 332, pp. 323 (1988); Verhoeyen et al., Science, 239, pp. 1534 (1988)). Accordingly, such "humanized" antibodies are chimeric antibodies (Cabilly et al., U.S. Pat. No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from the original antibody. In practice, humanized antibodies according to this invention are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in the original antibody.

[0067] The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity. According to the so-called "best-fit" method, the sequence of the variable domain of an antibody of this invention is screened against the entire library of known human variable-domain sequences. The human sequence which is closest to that of the mouse is then accepted as the human framework (FR) for the humanized antibody (Sims et al., J. Immunol., 151,

pp. 2296 (1993); Chothia and Lesk, J. Mol. Biol., 196, pp. 901 (1987)). Another method uses a particular framework from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework can be used for several different humanized antibodies (Carter et al., Proc. Natl. Acad. Sci. U.S.A., 89, pp. 4285 (1992); Presta et al., J. Immunol., 51, pp. 1993)).

[0068] It is further important that antibodies be humanized with retention of high affinity for multiple inhibitory KIR receptors and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the consensus and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding.

[0069] Another method of making "humanized" monoclonal antibodies is to use a XenoMouse® (Abgenix, Fremont, CA) as the mouse used for immunization. A XenoMouse is a murine host according to this invention that has had its immunoglobulin genes replaced by functional human immunoglobulin genes. Thus, antibodies produced by this mouse or in hybridomas made from the B cells of this mouse, are already humanized. The XenoMouse is described in United States Patent No. 6,162,963, which is herein incorporated in its entirety by reference. An analogous method can be achieved using a HuMAb-Mouse™ (Medarex).

[0070] Human antibodies may also be produced according to various other techniques,

such as by using, for immunization, other transgenic animals that have been engineered to express a human antibody repertoire (Jakobovitz et al., Nature 362 (1993) 255), or by selection of antibody repertoires using phage display methods. Such techniques are known to the skilled person and can be implemented starting from monoclonal antibodies as disclosed in the present application.

[0071] The antibodies of the present invention, preferably a DF-200-like antibody, may also be derivatized to "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in the original antibody, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (Cabilly et al., supra; Morrison et al., Proc. Natl. Acad. Sci. U.S.A., 81, pp. 6851 (1984)).

[0072] Other derivatives within the scope of this invention include functionalized antibodies, i.e., antibodies that are conjugated or covalently bound to a toxin, such as ricin, diphtheria toxin, abrin and *Pseudomonas* exotoxin; to a detectable moiety, such as a fluorescent moiety, a radioisotope or an imaging agent; or to a solid support, such as agarose beads or the like. Methods for conjugation or covalent bonding of these other agents to antibodies are well known in the art.

[0073] Conjugation to a toxin is useful for targeted killing of NK cells displaying one of the cross-reacting KIR receptors on its cell surface. Once the antibody of the invention binds to the cell surface of such cells, it is internalized and the toxin is released inside of the cell, selectively killing that cell. Such use is an alternate embodiment of the present invention.

[0074] Conjugation to a detectable moiety is useful when the antibody of this invention is used for diagnostic purposes. Such purposes include, but are not limited to, assaying biological samples for the presence of the NK cells bearing the cross-reacting KIR on their cell surface and detecting the presence of NK cells bearing the cross-reacting KIR

in a living organism. Such assay and detection methods are also alternate embodiments of the present invention.

[0075] Conjugation of an antibody of this invention to a solid support is useful as a tool for affinity purification of NK cells bearing the cross-reacting KIR on their cell surface from a source, such as a biological fluid. This method of purification is another alternate embodiment of the present invention, as is the resulting purified population of NK cells.

[0076] In an alternate embodiment, an antibody that binds a common determinant present on at least two different human inhibitory KIR receptor gene products, wherein said antibody is capable of neutralizing KIR-mediated inhibition of NK cell cytotoxicity on NK cells expressing at least one of said two different human inhibitory KIR receptors of this invention, including NKVSF1, may be incorporated into liposomes ("immunoliposomes"), alone or together with another substance for targeted delivery to an animal. Such other substances include nucleic acids for the delivery of genes for gene therapy or for the delivery of antisense RNA, RNAi or siRNA for suppressing a gene in an NK cell, or toxins or drugs for the targeted killing of NK cells.

Compositions and Administration

[0077] The invention also provides compositions that comprise an antibody, as well as fragments and derivatives thereof, wherein said antibody, fragment or derivative cross reacts with at least two inhibitory KIR receptors at the surface of NK cells, neutralizes their inhibitory signals and potentiates the activity of those cells, in any suitable vehicle in an amount effective to detectably potentiate NK cell cytotoxicity in a patient or in a biological sample comprising NK cells. The composition further comprises a pharmaceutically acceptable carrier. Such compositions are also referred to as "antibody compositions of this invention." The antibody NKVSF1 is included within the scope of antibodies that may be present in the antibody compositions of this invention.

[0078] The term "biological sample" as used herein includes but is not limited to a biological fluid (for example serum, lymph, blood), cell sample or tissue sample (for

example bone marrow).

[0079] Pharmaceutically acceptable carriers that may be used in these compositions include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat.

[0080] The compositions of this invention may be employed in a method of potentiating the activity of NK cells in a patient or a biological sample. This method comprises the step of contacting said composition with said patient or biological sample. Such method will be useful for both diagnostic and therapeutic purposes.

[0081] For use in conjunction with a biological sample, the antibody composition can be administered by simply mixing with or applying directly to the sample, depending upon the nature of the sample (fluid or solid). The biological sample may be contacted directly with the antibody in any suitable device (plate, pouch, flask, etc.). For use in conjunction with a patient, the composition must be formulated for administration to the patient.

[0082] The compositions of the present invention may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. The term "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional and intracranial injection or infusion techniques. Preferably, the compositions are administered orally, intraperitoneally or intravenously.

[0083] Sterile injectable forms of the compositions of this invention may be aqueous or

an oleaginous suspension. These suspensions may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or diglycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, such as carboxymethyl cellulose or similar dispersing agents that are commonly used in the formulation of pharmaceutically acceptable dosage forms including emulsions and suspensions. Other commonly used surfactants, such as Tweens, Spans and other emulsifying agents or bioavailability enhancers which are commonly used in the manufacture of pharmaceutically acceptable solid, liquid, or other dosage forms may also be used for the purposes of formulation.

[0084] The compositions of this invention may be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, aqueous suspensions or solutions. In the case of tablets for oral use, carriers commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried cornstarch. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening, flavoring or coloring agents may also be added.

[0085] Alternatively, the compositions of this invention may be administered in the form of suppositories for rectal administration. These can be prepared by mixing the agent with a suitable non-irritating excipient that is solid at room temperature but liquid at rectal temperature and therefore will melt in the rectum to release the drug. Such

materials include cocoa butter, beeswax and polyethylene glycols.

[0086] The compositions of this invention may also be administered topically, especially when the target of treatment includes areas or organs readily accessible by topical application, including diseases of the eye, the skin, or the lower intestinal tract. Suitable topical formulations are readily prepared for each of these areas or organs.

[0087] Topical application for the lower intestinal tract can be effected in a rectal suppository formulation (see above) or in a suitable enema formulation. Topically-transdermal patches may also be used.

[0088] For topical applications, the compositions may be formulated in a suitable ointment containing the active component suspended or dissolved in one or more carriers. Carriers for topical administration of the compounds of this invention include, but are not limited to, mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax and water. Alternatively, the compositions can be formulated in a suitable lotion or cream containing the active components suspended or dissolved in one or more pharmaceutically acceptable carriers. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

[0089] For ophthalmic use, the compositions may be formulated as micronized suspensions in isotonic, pH adjusted sterile saline, or, preferably, as solutions in isotonic, pH adjusted sterile saline, either with or without a preservative such as benzylalkonium chloride. Alternatively, for ophthalmic uses, the compositions may be formulated in an ointment such as petrolatum.

[0090] The compositions of this invention may also be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance

bioavailability, fluorocarbons, and/or other conventional solubilizing or dispersing agents.

[0091] Several monoclonal antibodies have been shown to be efficient in clinical situations, such as Rituxan (Rituximab), Herceptin (Trastuzumab) or Xolair (Omalizumab), and similar administration regimens (i.e., formulations and/or doses and/or administration protocols) may be used with the antibodies of this invention. Schedules and dosages for administration of the antibody in the pharmaceutical compositions of the present invention can be determined in accordance with known methods for these products, for example using the manufacturers' instructions. For example, an antibody present in a pharmaceutical composition of this invention can be supplied at a concentration of 10 mg/mL in either 100 mg (10 mL) or 500 mg (50 mL) single-use vials. The product is formulated for IV administration in 9.0 mg/mL sodium chloride, 7.35 mg/mL sodium citrate dihydrate, 0.7 mg/mL polysorbate 80, and Sterile Water for Injection. The pH is adjusted to 6.5. An exemplary suitable dosage range for an antibody in a pharmaceutical composition of this invention may between about 10 mg/m^2 and 500 mg/m^2. However, it will be appreciated that these schedules are exemplary and that an optimal schedule and regimen can be adapted taking into account the affinity and tolerability of the particular antibody in the pharmaceutical composition that must be determined in clinical trials. Quantities and schedule of injection of an antibody in a pharmaceutical composition of this invention that saturate NK cells for 24 hours, 48 hours 72 hours or a week or a month will be determined considering the affinity of the antibody and the its pharmacokinetic parameters.

[0092] According to another embodiment, the antibody compositions of this invention may further comprise another therapeutic agent, including agents normally utilized for the particular therapeutic purpose for which the antibody is being administered. The additional therapeutic agent will normally be present in the composition in amounts typically used for that agent in a monotherapy for the particular disease or condition being treated. Such therapeutic agents include, but are not limited to, therapeutic agents used in the treatment of cancers, therapeutic agents used to treat infectious disease, therapeutic agents used in other immunotherapies, cytokines (such as IL-2 or IL-15),

other antibodies and fragments of other antibodies.

[0093] For example, a number of therapeutic agents are available for the treatment of cancers. The antibody compositions and methods of the present invention may be combined with any other methods generally employed in the treatment of the particular disease, particularly a tumor, cancer disease, or other disease or disorder that the patient exhibits. So long as a particular therapeutic approach is not known to be detrimental to the patient's condition in itself, and does not significantly counteract the activity of the antibody in a pharmaceutical composition of this invention, its combination with the present invention is contemplated.

[0094] In connection with solid tumor treatment, the pharmaceutical compositions of the present invention may be used in combination with classical approaches, such as surgery, radiotherapy, chemotherapy, and the like. The invention therefore provides combined therapies in which a pharmaceutical composition of this invention is used simultaneously with, before, or after surgery or radiation treatment; or are administered to patients with, before, or after conventional chemotherapeutic, radiotherapeutic or anti-angiogenic agents, or targeted immunotoxins or coaguligands.

[0095] When one or more agents are used in combination with an antibody-containing composition of this invention in a therapeutic regimen, there is no requirement for the combined results to be additive of the effects observed when each treatment is conducted separately. Although at least additive effects are generally desirable, any increased anti-cancer effect above one of the single therapies would be of benefit. Also, there is no particular requirement for the combined treatment to exhibit synergistic effects, although this is certainly possible and advantageous.

[0096] To practice combined anti-cancer therapy, one would simply administer to an animal an antibody composition of this invention in combination with another anti-cancer agent in a manner effective to result in their combined anti-cancer actions within the animal. The agents would therefore be provided in amounts effective and for periods of time effective to result in their combined presence within the tumor vasculature and

their combined actions in the tumor environment. To achieve this goal, an antibody composition of this invention and anti-cancer agents may be administered to the animal simultaneously, either in a single combined composition, or as two distinct compositions using different administration routes.

[0097] Alternatively, the administration of an antibody composition of this invention may precede, or follow, the anti-cancer agent treatment by, e.g., intervals ranging from minutes to weeks and months. One would ensure that the anti-cancer agent and an antibody in the antibody composition of this invention exert an advantageously combined effect on the cancer.

[0098] Most anti-cancer agents would be given prior to an inhibitory KIR antibody composition of this invention in an anti-angiogenic therapy. However, when immunoconjugates of an antibody are used in the antibody composition of this invention, various anti-cancer agents may be simultaneously or subsequently administered.

[0099] In some situations, it may even be desirable to extend the time period for treatment significantly, where several days (2, 3, 4, 5, 6 or 7), several weeks (1, 2, 3, 4, 5, 6, 7 or 8) or even several months (1, 2, 3, 4, 5, 6, 7 or 8) lapse between the respective administration of the anti-cancer agent or anti-cancer treatment and the administration of an antibody composition of this invention. This would be advantageous in circumstances where the anti-cancer treatment was intended to substantially destroy the tumor, such as surgery or chemotherapy, and administration of an antibody composition of this invention was intended to prevent micrometastasis or tumor re-growth.

[00100] It also is envisioned that more than one administration of either an inhibitory KIR antibody-based composition of this invention or the anti-cancer agent will be utilized. These agents may be administered interchangeably, on alternate days or weeks; or a cycle of treatment with an inhibitory KIR antibody composition of this invention, followed by a cycle of anti-cancer agent therapy. In any event, to achieve tumor regression using a combined therapy, all that is required is to deliver both agents in a combined amount effective to exert an anti-tumor effect, irrespective of the times for

administration.

[00101] In terms of surgery, any surgical intervention may be practiced in combination with the present invention. In connection with radiotherapy, any mechanism for inducing DNA damage locally within cancer cells is contemplated, such as gamma-irradiation, X-rays, UV-irradiation, microwaves and even electronic emissions and the like. The directed delivery of radioisotopes to cancer cells is also contemplated, and this may be used in connection with a targeting antibody or other targeting means.

[00102] In other aspects, immunomodulatory compounds or regimens may be administered in combination with or as part of the antibody compositions of the present invention. Preferred examples of immunomodulatory compounds include cytokines. Various cytokines may be employed in such combined approaches. Examples of cytokines useful in the combinations contemplated by this invention include IL-1alpha IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-15, IL-21, TGF-beta, GM-CSF, M-CSF, G-CSF, TNF-alpha, TNF-beta, LAF, TCGF, BCGF, TRF, BAF, BDG, MP, LIF, OSM, TMF, PDGF, IFN-alpha, IFN-beta, IFN-gamma. Cytokines used in the combination treatment or compositions of this invention are administered according to standard regimens, consistent with clinical indications such as the condition of the patient and relative toxicity of the cytokine.

[00103] In certain embodiments, the cross-reacting inhibitory KIR antibody-comprising therapeutic compositions of the present invention may be administered in combination with or may further comprise a chemotherapeutic or hormonal therapy agent. A variety of hormonal therapy and chemotherapeutic agents may be used in the combined treatment methods disclosed herein. Chemotherapeutic agents contemplated as exemplary include, but are not limited to, alkylating agents, antimetabolites, cytotoxic antibiotics, vinca alkaloids, for example adriamycin, dactinomycin, mitomycin, carminomycin, daunomycin, doxorubicin, tamoxifen, taxol, taxotere, vincristine, vinblastine, vinorelbine, etoposide (VP-16), 5-fluorouracil (5FU), cytosine arabinoside, cyclophosphamide, thiotepa, methotrexate, camptothecin, actinomycin-D, mitomycin C,

cisplatin (CDDP), aminopterin, combretastatin(s) and derivatives and prodrugs thereof.

[00104] Hormonal agents include, but are not limited to, for example LHRH agonists such as leuprorelin, goserelin, triptorelin, and buserelin; anti-estrogens such as tamoxifen and toremifene; anti-androgens such as flutamide, nilutamide, cyproterone and bicalutamide; aromatase inhibitors such as anastrozole, exemestane, letrozole and fadrozole; and progestagens such as medroxy, chlormadinone and megestrol.

[00105] As will be understood by those of ordinary skill in the art, the appropriate doses of chemotherapeutic agents will approximate those already employed in clinical therapies wherein the chemotherapeutics are administered alone or in combination with other chemotherapeutics. By way of example only, agents such as cisplatin, and other DNA alkylating may be used. Cisplatin has been widely used to treat cancer, with efficacious doses used in clinical applications of 20 mg/m^2 for 5 days every three weeks for a total of three courses. Cisplatin is not absorbed orally and must therefore be delivered via injection intravenously, subcutaneously, intratumorally or intraperitoneally.

[00106] Further useful chemotherapeutic agents include compounds that interfere with DNA replication, mitosis and chromosomal segregation, and agents that disrupt the synthesis and fidelity of polynucleotide precursors. A number of exemplary chemotherapeutic agents for combined therapy are listed in Table C of U.S. Patent No. 6,524,583, the disclosure of which agents and indications are specifically incorporated herein by reference. Each of the agents listed are exemplary and not limiting. The skilled artisan is directed to "Remington's Pharmaceutical Sciences" 15th Edition, chapter 33, in particular pages 624-652. Variation in dosage will likely occur depending on the condition being treated. The physician administering treatment will be able to determine the appropriate dose for the individual subject.

[00107] The present cross-reacting inhibitory KIR antibody compositions of this invention may be used in combination with any one or more other anti-angiogenic therapies or may further comprise anti-angiogenic agents. Examples of such agents

include neutralizing antibodies, antisense RNA, siRNA, RNAi, RNA aptamers and ribozymes each directed against VEGF or VEGF receptors (U.S. Patent No. 6,524,583, the disclosure of which is incorporated herein by reference). Variants of VEGF with antagonistic properties may also be employed, as described in WO 98/16551, specifically incorporated herein by reference. Further exemplary anti-angiogenic agents that are useful in connection with combined therapy are listed in Table D of U.S. Patent No. 6,524,583, the disclosure of which agents and indications are specifically incorporated herein by reference.

[00108] The inhibitory KIR antibody compositions of this invention may also be advantageously used in combination with methods to induce apoptosis or may comprise apoptotic agents. For example, a number of oncogenes have been identified that inhibit apoptosis, or programmed cell death. Exemplary oncogenes in this category include, but are not limited to, bcr-abl, bcl-2 (distinct from bcl-1, cyclin D1; GenBank accession numbers M14745, X06487; U.S. Pat. Nos. 5,650,491; and 5,539,094; each incorporated herein by reference) and family members including Bcl-x1, Mcl-1, Bak, A1, and A20. Overexpression of bcl-2 was first discovered in T cell lymphomas. The oncogene bcl-2 functions by binding and inactivating Bax, a protein in the apoptotic pathway. Inhibition of bcl-2 function prevents inactivation of Bax, and allows the apoptotic pathway to proceed. Inhibition of this class of oncogenes, e.g., using antisense nucleotide sequences, RNAi, siRNA or small molecule chemical compounds, is contemplated for use in the present invention to give enhancement of apoptosis (U.S. Pat. Nos. 5,650,491; 5,539,094; and 5,583,034; each incorporated herein by reference).

[00109] The inhibitory KIR antibody compositions of this invention may also comprise or be used in combination with molecules that comprise a targeting portion, e.g., antibody, ligand, or conjugate thereof, directed to a specific marker of a target cell ("targeting agent"), for example a target tumor cell. Generally speaking, targeting agents for use in these additional aspects of the invention will preferably recognize accessible tumor antigens that are preferentially, or specifically, expressed in the tumor site. The targeting agents will generally bind to a surface-expressed, surface-accessible or surface-localized component of a tumor cell. The targeting agents will also preferably exhibit

properties of high affinity; and will not exert significant in vivo side effects against life-sustaining normal tissues, such as one or more tissues selected from heart, kidney, brain, liver, bone marrow, colon, breast, prostate, thyroid, gall bladder, lung, adrenals, muscle, nerve fibers, pancreas, skin, or other life-sustaining organ or tissue in the human body. The term "not exert significant side effects," as used herein, refers to the fact that a targeting agent, when administered in vivo, will produce only negligible or clinically manageable side effects, such as those normally encountered during chemotherapy.

[00110] In the treatment of tumors, an antibody composition of this invention may additionally comprise or may be used in combination with adjunct compounds. Adjunct compounds may include by way of example anti-emetics such as serotonin antagonists and therapies such as phenothiazines, substituted benzamides, antihistamines, butyrophenones, corticosteroids, benzodiazepines and cannabinoids; bisphosphonates such as zoledronic acid and pamidronic acid; and hematopoietic growth factors such as erythropoietin and G-CSF, for example filgrastim, lenograstim and darbepoietin.

[00111] In another embodiment, two or more antibodies of this invention having different cross-reactivities, including NKVSF1, may be combined in a single composition so as to neutralize the inhibitory effects of as many inhibitory KIR gene products as possible. Compositions comprising combinations of cross-reactive inhibitory KIR antibodies of this invention, or fragments or derivatives thereof, will allow even wider utility because there likely exists a small percentage of the human population that may lack each of the inhibitory KIR gene products recognized by a single cross-reacting antibody. Similarly, an antibody composition of this invention may further comprise one or more antibodies that recognize single inhibitory KIR subtypes. Such combinations would again provide wider utility in a therapeutic setting.

[00112] The invention also provides a method of potentiating NK cell activity in a patient in need thereof, comprising the step of administering a composition according to this invention to said patient. The method is more specifically directed at increasing NK cell activity in patients having a disease in which increased NK cell activity is beneficial, which involves, affects or is caused by cells susceptible to lysis by NK cells, or which is

caused or characterized by insufficient NK cell activity, such as a cancer, another proliferative disorder, an infectious disease or an immune disorder. More specifically, the methods of the present invention are utilized for the treatment of a variety of cancers and other proliferative diseases including, but not limited to, carcinoma, including that of the bladder, breast, colon, kidney, liver, lung, ovary, prostate, pancreas, stomach, cervix, thyroid and skin, including squamous cell carcinoma; hematopoietic tumors of lymphoid lineage, including leukemia, acute lymphocytic leukemia, acute lymphoblastic leukemia, B-cell lymphoma, T-cell lymphoma, Hodgkins lymphoma, non-Hodgkins lymphoma, hairy cell lymphoma and Burketts lymphoma; hematopoietic tumors of myeloid lineage, including acute and chronic myelogenous leukemias and promyelocytic leukemia; tumors of mesenchymal origin, including fibrosarcoma and rhabdomyoscarcoma; other tumors, including melanoma, seminoma, teratocarcinoma, neuroblastoma and glioma; tumors of the central and peripheral nervous system, including astrocytoma, neuroblastoma, glioma, and schwannomas; tumors of mesenchymal origin, including fibrosarcoma, rhabdomyoscaroma, and osteosarcoma; and other tumors, including melanoma, xeroderma pigmentosum, keratoacanthoma, seminoma, thyroid follicular cancer and teratocarcinoma.

[00113] Preferred disorders that can be treated according to the invention include hematopoietic tumors of lymphoid lineage, for example T-cell and B-cell tumors, including but not limited to T-cell disorders such as T-prolymphocytic leukemia (T-PLL), including of the small cell and cerebriform cell type; large granular lymphocyte leukemia (LGL) preferably of the T-cell type; Sezary syndrome (SS); Adult T-cell leukemia lymphoma (ATLL); a/d T-NHL hepatosplenic lymphoma; peripheral/post-thymic T cell lymphoma (pleomorphic and immunoblastic subtypes); angio immunoblastic T-cell lymphoma; angiocentric (nasal) T-cell lymphoma; anaplastic (Ki 1+) large cell lymphoma; intestinal T-cell lymphoma; T-lymphoblastic; and lymphoma/leukaemia (T-Lbly/T-ALL).

[00114] Other proliferative disorders can also be treated according to the invention, including for example hyperplasias, fibrosis (especially pulmonary, but also other types of fibrosis, such as renal fibrosis), angiogenesis, psoriasis, atherosclerosis and smooth

muscle proliferation in the blood vessels, such as stenosis or restenosis following angioplasty. The cross-reacting inhibitory KIR antibody of this invention can be used to treat or prevent infectious diseases, including preferably any infections caused by viruses, bacteria, protozoa, molds or fungi. Such viral infectious organisms include, but are not limited to, hepatitis type A, hepatitis type B, hepatitis type C, influenza, varicella, adenovirus, herpes simplex type I (HSV-1), herpes simplex type 2 (HSV-2), rinderpest, rhinovirus, echovirus, rotavirus, respiratory syncytial virus, papilloma virus, papilloma virus, cytomegalovirus, echinovirus, arbovirus, huntavirus, coxsackie virus, mumps virus, measles virus, rubella virus, polio virus and human immunodeficiency virus type I or type 2 (HIV-1, HIV-2).

[00115] Bacterial infections that can be treated according to this invention include, but are not limited to, infections caused by the following: Staphylococcus; Streptococcus, including S. pyogenes; Enterococcl; Bacillus, including Bacillus anthracis, and Lactobacillus; Listeria; Corynebacterium diphtheriae; Gardnerella including G. vaginalis; Nocardia; Streptomyces; Thermoactinomyces vulgaris; Treponerna; Camplyobacter, Pseudomonas including Raeruginosa; Legionella; Neisseria including N.gonorrhoeae and N.meningitides; Flavobacterium including F. meningosepticum and F. odoraturn; Brucella; Bordetella including B. pertussis and B. bronchiseptica; Escherichia including E. coli, Klebsiella; Enterobacter, Serratia including S. marcescens and S. liquefaciens; Edwardsiella; Proteus including P. mirabilis and P. vulgaris; Streptobacillus; Rickettsiaceae including R. fickettsfi, Chlamydia including C. psittaci and C. trachornatis; Mycobacterium including M. tuberculosis, M. intracellulare, M. folluiturn, M. laprae, M. avium, M. bovis, M. africanum, M. kansasii, M. intracellulare, and M. lepraemurium; and Nocardia.

[00116] Protozoa infections that may be treated according to this invention include, but are not limited to, infections caused by leishmania, kokzidioa, and trypanosoma. A complete list of infectious diseases can be found on the website of the National Center for Infectious Disease (NCID) at the Center for Disease Control (CDC) (http://www.cdc.gov/ncidod/diseases/), which list is incorporated herein by reference. All of said diseases are candidates for treatment using the cross-reacting inhibitory KIR

antibodies of the invention.

[00117] Such methods of treating various infectious diseases may employ the antibody composition of this invention, either alone or in combination with other treatments and/or therapeutic agents known for treating such diseases, including anti-viral agents, anti-fungal agents, antibacterial agents, antibiotics, anti-parasitic agents and anti-protozoal agents. When these methods involve additional treatments with additional therapeutic agents, those agents may be administered together with the antibodies of this invention as either a single dosage form or as separate, multiple dosage forms. When administered as a separate dosage form, the additional agent may be administered prior to, simultaneously with, of following administration of the antibody of this invention.

[00118] Further aspects and advantages of this invention will be disclosed in the following experimental section, which should be regarded as illustrative and not limiting the scope of this application.

Example 1

Purification of PBLs and generation of polyclonal or clonal NK cell lines.

[00119] PBLs were derived from healthy donors by Ficoll Hypaque gradients and depletion of plastic adherent cells. To obtain enriched NK cells, PBLs were incubated with anti CD3, anti CD4 and anti HLA-DR mAbs (30 minutes at 4°C), followed by goat anti mouse magnetic beads (Dynal) (30 minutes at 4°C) and immunomagnetic selection by methods known in the art (Pende et al., 1999). $CD3^-$, $CD4^-$, DR^- cells were cultivated on irradiated feeder cells and 100 U/ml Interleukin 2 (Proleukin, Chiron Corporation) and 1.5 ng/ml Phytohemagglutinin A (Gibco BRL) to obtain polyclonal NK cell populations. NK cells were cloned by limiting dilution and clones of NK cells were characterized by flow cytometry for expression of cell surface receptors.

[00120] The mAbs used were JT3A (IgG2a, anti CD3), EB6 and GL183 (IgG1 anti KIR2DL1 and KIR2DL3 respectively), XA-141 IgM (anti KIR2DL1 with the same specificity as EB6), anti CD4 (HP2.6), and anti DR (D1.12, IgG2a). Instead of JT3A,

HP2.6, and DR1.12, which were produced by applicants, commercially available mAbs of the same specificities can be used (Beckman Coulter Inc., Fullerton, CA). EB6 and GL183 are commercially available (Beckman Coulter Inc., Fullerton, CA. XA-141 is not commercially available, but EB6 can be used for control reconstitution of lysis as described in (Moretta et al., 1993).

[00121] Cells were stained with the appropriate antibodies (30mns at 4°C) followed by PE or FITC conjugated polyclonal anti mouse antibodies (Southern Biotechnology Associates Inc). Samples were analyzed by cytofluorometric analysis on a FACSAN apparatus (Becton Dickinson, Mountain View, CA).

[00122] The following clones were used in this study. CP11, CN5 and CN505 are KIR2DL1 positive clones and are stained by EB6 ((IgG1 anti KIR2DL1) or XA-141 (IgM anti KIR2DL1 with same specificity as compared to EB6 antibodies). CN12 and CP502 are KIR2DL3 positive clones and are stained by GL183 antibody (IgG1 anti KIR2DL3).

[00123] The cytolytic activity of NK clones was assessed by a standard 4 hour ^{51}Cr release assay in which effector NK cells were tested on Cw3 or Cw4 positive cell lines known for their sensitivity to NK cell lysis. All the targets were used at 5000 cells per well in microtitration plate and the effector:target ratio is indicated in the Figures (usually 4 effectors per target cells). The cytolytic assay was performed with or without supernatant of the indicated monoclonal antibodies at a ½ dilution. The procedure was essentially the same as described in (Moretta et al., 1993).

Example 2

Generation of new mAbs

[00124] mAbs were generated by immunizing 5 week old Balb C mice with activated polyclonal or monoclonal NK cell lines as described in (Moretta et al., 1990). After different cell fusions, the mAbs were first selected for their ability to cross react with EB6 and GL183 positive NK cell lines and clones. Positive monoclonal antibodies were further screened for their ability to reconstitute lysis by EB6 positive or GL183 positive NK clones of Cw4 or Cw3 positive targets respectively.

[00125] One of the monoclonal antibodies, the DF200 mAb, was found to react with various members of the KIR family including KIR2DL1, KIR2DL2/3. Regarding the staining of NK cells with DF200mAb both KIR2DL1+ and KIR2DL2/3+ cells were stained brightly (Figure 1).

[00126] NK clones expressing one or another (or even both) of these HLA class I-specific inhibitory receptors were used as effectors cells against target cells expressing one or more HLA-C alleles. As expected, KIR2DL1$^+$ NK clones displayed little if any cytolytic activity against target cells expressing HLA-Cw4 and KIR2DL3$^+$ NK clones displayed little or no activity on Cw3 positive targets. However, in the presence of DF200mAb (used to mask their KIR2DL receptors) NK clones became unable to recognize their HLA-C ligands and displayed strong cytolytic activity on Cw3 or Cw4 targets.

[00127] For example, the C1R cell line (CW4$^+$ EBV cell line, ATCC n°CRL 1993) was not killed by KIR2DL1$^+$ NK clones (CN5/CN505), but the inhibition could be efficiently reversed by the use of either DF200 or a conventional anti KIR2DL1 mAb. On the other hand NK clones expressing the KIR2DL2/3$^+$ KIR2DL1$^-$ phenotype (CN12) efficiently killed C1R cells and this killing was unaffected by the DF200mAb (Figure 2). Similar results are obtained with KIR2DL2- or KIR2DL3-positive NK clones on Cw3 positive targets.

Example 3

Biacore analysis of DF200 mAb/KIR2DL1 and DF200 mAb/KIR2DL3 interactions

Production and purification of recombinant proteins

[00128] The KIR2DL1 and KIR2DL3 recombinant proteins were produced in *E. coli*. cDNA encoding the entire extracellular domain of KIR2DL1 and KIR2DL3 were amplified by PCR from pCDM8 clone 47.11 vector (Biassoni et al, 1993) and RSVS(gpt)183 clone 6 vector (Wagtman et al, 1995) respectively, using the following primers:

Sense: 5'-GGAATTCCAGGAGGAATTTAAAATGCATGAGGGAGTCCACAG-3'
Anti-sense: 5'- CGGGATCCCAGGTGTCTGGGGTTACC -3'
They were cloned into the pML1 expression vector in frame with a sequence encoding a biotinylation signal (Saulquin et al, 2003).

[00129] Protein expression was performed in the BL21(DE3) bacterial strain (Invitrogen). Transfected bacteria were grown to OD_{600}=0.6 at 37°C in medium supplemented with ampicillin (100 µg/ml) and expression was induced with 1 mM IPTG.

[00130] Proteins were recovered from inclusion bodies under denaturing conditions (8 M urea). Refolding of the recombinant proteins was performed in 20 mM Tris, pH 7.8, NaCl 150 mM buffer containing L-arginine (400 mM, Sigma) and β-mercaptoethanol (1 mM), at room temperature, by decreasing the urea concentration in a six step dialysis (4, 3, 2, 1 0.5 and 0 M urea, respectively). Reduced and oxidized glutathione (5 mM and 0.5 mM respectively, Sigma) were added during the 0.5 and 0 M urea dialysis steps. Finally, the proteins were dialyzed extensively against 10 mM Tris, pH 7.5, NaCl 150 mM buffer. Soluble, refolded proteins were concentrated and then purified on a Superdex 200 size-exclusion column (Pharmacia; AKTA system).

[00131] Surface plasmon resonance measurements were performed on a Biacore apparatus (Biacore). In all Biacore experiments HBS buffer supplemented with 0.05% surfactant P20 served as running buffer.

Protein immobilisation.

[00132] Recombinant KIR2DL1 and KIR2DL3 proteins produced as described above were immobilized covalently to carboxyl groups in the dextran layer on a Sensor Chip CM5 (Biacore). The sensor chip surface was activated with EDC/NHS (N-ethyl-N'-(3-dimethylaminopropyl)carbodiimidehydrochloride and N-hydroxysuccinimide, Biacore). Proteins, in coupling buffer (10 mM acetate, pH 4.5) were injected. Deactivation of the remaining activated groups was performed using 100 mM ethanolamine pH 8 (Biacore).

Affinity measurements.

[00133] For kinetic measurements, various concentrations of the soluble antibody (1 x 10^{-7} to 4 x 10^{-10} M) were applied onto the immobilized sample. Measurements were performed at a 20 µl/min continuous flow rate. For each cycle, the surface of the sensor chip was regenerated by 5 µl injection of 10 mM NaOH pH 11. The BIAlogue Kinetics Evaluation program (BIAevaluation 3.1, Biacore) was used for data analysis. The soluble analyte (40 µl at various concentrations) was injected at a flow rate of 20 µl/min in HBS buffer, on dextran layers containing 500 or 540 reflectance units (RU), and 1000 or 700 RU of KIR2DL1 and KIR2DL3, respectively. Data are representative of 6 independent experiments. The results are shown in Table 1, below.

[00134] Table 1. BIAcore analysis of DF200 mAb binding to immobilized KIR2DL1 and KIR2DL3.

Protein	K_D (10^{-9} M)
KIR2DL1	10.9 +/- 3.8
KIR2DL3	2.0 +/- 1.9

K_D: Dissociation constant.

REFERENCES

Moretta, A., Bottino, C., Pende, D., Tripodi, G., Tambussi, G., Viale, O., Orengo, A., Barbaresi, M., Merli, A., Ciccone, E., and et al. (1990). Identification of four subsets of human CD3-CD16+ natural killer (NK) cells by the expression of clonally distributed functional surface molecules: correlation between subset assignment of NK clones and ability to mediate specific alloantigen recognition. J Exp Med *172*, 1589-1598.

Moretta, A., Vitale, M., Bottino, C., Orengo, A. M., Morelli, L., Augugliaro, R., Barbaresi, M., Ciccone, E., and Moretta, L. (1993). P58 molecules as putative receptors for major histocompatibility complex (MHC) class I molecules in human natural killer (NK) cells. Anti-p58 antibodies reconstitute lysis of MHC class I-protected cells in NK clones displaying different specificities. J Exp Med *178*, 597-604.

Pende, D., Parolini, S., Pessino, A., Sivori, S., Augugliaro, R., Morelli, L., Marcenaro, E., Accame, L., Malaspina, A., Biassoni, R., *et al.* (1999). Identification and molecular characterization of NKp30, a novel triggering receptor involved in natural cytotoxicity mediated by human natural killer cells. J Exp Med *190*, 1505-1516.

Ruggeri, L., Capanni, M., Urbani, E., Perruccio, K., Shlomchik, W. D., Tosti, A., Posati, S., Rogaia, D., Frassoni, F., Aversa, F., *et al.* (2002). Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science *295*, 2097-2100.

Wagtmann N, Biassoni R, Cantoni C, Verdiani S, Malnati MS, Vitale M, Bottino C, Moretta L, Moretta A, Long EO.Molecular clones of the p58 NK cell receptor reveal immunoglobulin-related molecules with diversity in both the extra- and intracellular domains.Immunity. 1995 May;2(5):439-49.

Biassoni R, Verdiani S, Cambiaggi A, Romeo PH, Ferrini S, Moretta L.Human CD3-CD16+ natural killer cells express the hGATA-3 T cell transcription factor and an unrearranged 2.3-kb TcR delta transcript. Eur J Immunol. 1993 May;23(5):1083-7.

Saulquin X, Gastinel LN, Vivier E.Crystal structure of the human natural killer cell activating receptor KIR2DS2 (CD158j) J Exp Med. 2003 Apr 7;197(7):933-8.

CLAIMS

1. An antibody that binds a common determinant present on at least two different human inhibitory KIR receptor gene products, wherein said antibody is capable of neutralizing KIR-mediated inhibition of NK cell cytotoxicity on NK cells expressing at least one of said two different human inhibitory KIR receptors.

2. The antibody according to claim 1, wherein said antibody is not NKVSF1

3. The antibody of claim 1, wherein said antibody binds a common determinant present on KIR2DL1 and KIR2DL2/3.

4. The antibody of claim 3, wherein said antibody inhibits the binding of a HLA-C allele molecule having a Lys residue at position 80 to a human KIR2DL1 receptor, and the binding of a HLA-C allele molecule having an Asn residue at position 80 to human KIR2DL2/3 receptors.

5. The antibody of claim 4, wherein said antibody binds to substantially the same epitope as monoclonal antibody DF200 produced by hybridoma DF200.

6. The antibody of claim 5, wherein said antibody is a monoclonal antibody or a fragment of a monoclonal antibody.

7. The antibody of claim 6, wherein said antibody is monoclonal antibody DF200 produced by hybridoma DF200.

8. The antibody of claim 1, wherein said antibody is an antibody fragment selected from Fab, Fab', Fab'-SH, F(ab')2, Fv, diabodies, single-chain antibody fragment, or a multispecific antibody formed from antibody fragments.

9. The antibody of claim 6, wherein said antibody is humanized or a chimeric antibody.

10. A hybridoma comprising:

a) a B cell from a non-human mammalian host that has been immunized with an antigen that comprises an epitope present on an inhibitory KIR polypeptide, fused to

b) an immortalized cell,

wherein said hybridoma produces a monoclonal antibody binds a common determinant present on at least two different human inhibitory KIR receptor gene products, wherein said antibody is capable of neutralizing KIR-mediated inhibition of NK cell cytotoxicity on a population of NK cells expressing said at least two different human inhibitory KIR receptor gene products.

11. The hybridoma according to claim 10, wherein said hybridoma does not produce monoclonal antibody NKVSF1.

12. The hybridoma of claim 10, wherein said common determinant is present on KIR2DL1 and KIR2DL2/3.

13. The hybridoma of claim 12, wherein said hybridoma produces an antibody that inhibits the binding of a HLA-c allele molecule having a Lys residue at position 80 to a human KIR2DL1 receptor, and the binding of a HLA-C allele molecule having an Asn residue at position 80 to human KIR2DL2/3 receptors.

14. The hybridoma of claim 13, wherein said hybridoma produces an antibody that binds to substantially the same epitope as monoclonal antibody DF200 produced by hybridoma DF200.

15. The hybridoma of claim 14, wherein said hybridoma is DF200.

16. A method of producing an antibody that binds a common determinant present on at least two different human inhibitory KIR receptor gene products, wherein said antibody is capable of neutralizing KIR-mediated inhibition of NK cell cytotoxicity on a

population of NK cells expressing said at least two different human inhibitory KIR receptor gene products, said method comprising the steps of:

a) immunizing a non-human mammal with an immunogen comprising an inhibitory KIR polypeptide;

b) preparing antibodies from said immunized animal, wherein said antibodies bind said KIR polypeptide,

c) selecting antibodies of (b) that cross react with at least two different human inhibitory KIR receptor gene products, and

d) selecting antibodies of (c) that capable of neutralizing KIR-mediated inhibition of NK cell cytotoxicity on a population of NK cells expressing said at least two different human inhibitory KIR receptor gene products,

wherein the order of steps (c) and (d) is optionally reversed.

17. The method of claim 16, wherein said antibody selected in step c) or d) is not NKVSF1.

18. The method of claim 16, wherein the antibody prepared in step (b) is a monoclonal antibody.

19. The method of claim 16, wherein the inhibitory KIR polypeptide used for immunization is a KIR2DL polypeptide and the antibodies selected in step (c) cross-react with at least KIR2DL1 and KIR2DL2/3.

20. The method of claim 19, wherein said antibody selected in step (c) inhibits the binding of a HLA-c allele molecule having a Lys residue at position 80 to a human KIR2DL1 receptor, and the binding of a HLA-C allele molecule having an Asn residue at position 80 to human KIR2DL2/3 receptors.

21. The method of claim 16, wherein the antibodies selected in step (d) cause at least a 50% potentiation in NK cytotoxicity.

22. The method according to claim 16, wherein said antibody or antibody fragment binds to substantially the same epitope as monoclonal antibody DF200.

23. The method of claim 18, comprising the additional step of making fragments of the selected monoclonal antibodies.

24. A method of producing an antibody that binds a common determinant present on at least two different human inhibitory KIR receptor gene products, wherein said antibody is capable of neutralizing KIR-mediated inhibition of NK cell cytotoxicity on a population of NK cells expressing said at least two different human inhibitory KIR receptor gene products, said method comprising the steps of:

a) selecting, from a library or repertoire, a monoclonal antibody or an antibody fragment that cross reacts with at least two different human inhibitory KIR2DL receptor gene products, and

b) selecting an antibody of (a) that capable of neutralizing KIR-mediated inhibition of NK cell cytotoxicity on a population of NK cells expressing said at least two different human inhibitory KIR2DL receptor gene products.

25. The method according to claim 24, wherein said antibody selected in step b) is not NKVSF1.

26. The method of claim 24, wherein the antibody selected in step (b) inhibits the binding of a HLA-c allele molecule having a Lys residue at position 80 to a human KIR2DL1 receptor, and the binding of a HLA-C allele molecule having an Asn residue at position 80 to human KIR2DL2/3 receptors.

27. The method of claim 24, wherein the antibody selected in step (b) causes at least a 50% potentiation in NK cytotoxicity.

28. The method according to claim 24, wherein said antibody binds to substantially the same epitope as monoclonal antibody DF200.

29. The method of claim 24, comprising the additional step of making fragments of the selected monoclonal antibodies.

30. A method of producing an antibody that binds a common determinant present on at least two different human inhibitory KIR receptor gene products, wherein said antibody is capable of neutralizing KIR-mediated inhibition of NK cell cytotoxicity on a population of NK cells expressing said at least two different human inhibitory KIR receptor gene products, said method comprising the steps of:

a) culturing a hybridoma of any one of claim 10 to 15 under conditions that cause the expression of said monoclonal antibody; and

b) separating said monoclonal antibody from said hybridoma.

31. The method of claim 30, comprising the additional step of making fragments of the said monoclonal antibody.

32. A method of producing an antibody that binds a common determinant present on at least two different human inhibitory KIR receptor gene products, wherein said antibody is capable of neutralizing KIR-mediated inhibition of NK cell cytotoxicity on a population of NK cells expressing said at least two different human inhibitory KIR receptor gene products, said method comprising the steps of:

a) isolating from a hybridoma of any one of claim 10 to 15 DNA encoding said monoclonal antibody;

b) optionally modifying said DNA so as to encode for a modified or derivatized antibody selected from a humanized antibody, a chimeric antibody, a single chain antibody or an immunoreactive fragment of an antibody;

c) inserting said DNA or modified DNA into an expression vector, wherein said antibody or antibody fragment is capable of being expressed when said expression vector is present in a host grown under appropriate conditions;

d) transfecting a host cell with said expression vector, wherein said host cell does not otherwise produce immunoglobulin protein;

e) culturing said transfected host cell under conditions which cause the expression of said antibody or antibody fragment; and

f) isolating the antibody or antibody fragment produced by said transfected host cell.

33. A composition comprising an antibody that binds a common determinant present on at least two different human inhibitory KIR receptor gene products, wherein said antibody is capable of neutralizing KIR-mediated inhibition of NK cell cytotoxicity on NK cells expressing at least one of said two different human inhibitory KIR receptors, said antibody being present in an amount effective to detectably potentiate NK cell cytotoxicity in a patient or in a biological sample comprising NK cells; and a pharmaceutically acceptable carrier or excipient.

34. The composition of claim 33, further comprising a therapeutic agent selected from an immunomodulatory agent, a hormonal agent, a chemotherapeutic agent, an anti-angiogenic agent, an apoptotic agent, a second antibody that binds to and inhibits an inhibitory KIR receptor, an anti -infective agent, a targeting agent or an adjunct compound.

35. The composition of claim 34, wherein said immunomodulatory agent is selected from IL-1alpha IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-15, IL-21, TGF-beta, GM-CSF, M-CSF, G-CSF, TNF-alpha, TNF-beta, LAF, TCGF, BCGF, TRF, BAF, BDG, MP, LIF, OSM, TMF, PDGF, IFN-alpha, IFN-beta, or IFN-gamma.

36. The composition of claim 34, wherein said chemotherapeutic agent is selected from alkylating agents, antimetabolites, cytotoxic antibiotics, adriamycin, dactinomycin, mitomycin, carminomycin, daunomycin, doxorubicin, tamoxifen, taxol, taxotere, vincristine, vinblastine, vinorelbine, etoposide (VP-16), 5-fluorouracil (5FU), cytosine arabinoside, cyclophosphamide, thiotepa, methotrexate, camptothecin, actinomycin-D, mitomycin C, cisplatin (CDDP), aminopterin, combretastatin(s), other vinca alkyloids and derivatives or prodrugs thereof.

37. The composition of claim 34, wherein said hormonal agent is selected from leuprorelin, goserelin, triptorelin, buserelin, tamoxifen, toremifene, flutamide, nilutamide, cyproterone bicalutamid anastrozole, exemestane, letrozole, fadrozole medroxy, chlormadinone, megestrol, other LHRH agonists, other anti-estrogens, other anti-androgens, other aromatase inhibitors, or other progestagens.

38. The composition of claim 34, wherein said adjunct compound is selected from phenothiazines, substituted benzamides, antihistamines, butyrophenones, corticosteroids, benzodiazepines, cannabinoids, zoledronic acid, pamidronic acid, erythropoietin, G-CSF, filgrastim, lenograstim, darbepoietin other anti-emetics, other serotonin antagonists, other bisphosphonatesor other hematopoietic growth factors.

39. The composition of claim 34, wherein said anti-apoptotic agents is an antisense nucleotide sequence, RNAi, siRNA or small molecule chemical compound that inhibits the expression of a gene selected from bcr-abl, bcl-2, Bcl-x1, Mcl-1, Bak, A1, or A20.

40. The composition of claim 34, wherein said anti-angiogenic agent is selected from neutralizing antibodies, antisense RNA, siRNA, RNAi, RNA aptamers or ribozymes directed against a gene encoding VEGF, a gene encoding a VEGF receptors, VEGF, or a VEGF receptor ; or a variant of VEGF possessing antagonistic properties against VEGF.

41. The composition of claim 34, wherein said second antibody that binds to and inhibits an inhibitory KIR receptor is an antibody or a derivative or fragment thereof that binds to an epitope of an inhibitory KIR receptor that differs from the epitope bound by said antibody that binds a common determinant present on at least two different human inhibitory KIR receptor gene products.

42. A method of potentiating NK cell activity in a patient in need thereof, comprising the step of administering to said patient a composition of claim 33.

43. The method of claim 42, wherein said patient is suffering from cancer, another proliferative disorder, an infectious disease or an immune disorder.

44. The method of claim 43, wherein said patient is suffering from squamous cell carcinoma, leukemia, acute lymphocytic leukemia, acute lymphoblastic leukemia, B-cell lymphoma, T-cell lymphoma, Hodgkins lymphoma, non-Hodgkins lymphoma, hairy cell lymphoma, Burketts lymphoma, acute or chronic myelogenous leukemias, promyelocytic leukemia, fibrosarcoma, rhabdomyoscarcoma; melanoma, seminoma, teratocarcinoma, neuroblastoma, glioma, astrocytoma, neuroblastoma, glioma, schwannomas; fibrosarcoma, rhabdomyoscaroma, osteosarcoma, melanoma, xeroderma pigmentosum, keratoacanthoma, seminoma, thyroid follicular cancer, teratocarcinoma, other carcinoma of the bladder, breast, colon, kidney, liver, lung, ovary, prostate, pancreas, stomach, cervix, thyroid or skin, other hematopoietic tumors of lymphoid lineage, other hematopoietic tumors of myeloid lineage, other tumors of mesenchymal origin, other tumors of the central or peripheral nervous system, or other tumors of mesenchymal origin.

45. The method according to claim 44, wherein said patient is suffering from a hematopoietic tumor of lymphoid lineage.

46. The method according to claim 45, wherein said tumor is selected from T-prolymphocytic leukemia (T-PLL) including of the small cell and cerebriform cell type; large granular lymphocyte leukemia (LGL) of the T-cell type; Sezary syndrome (SS); adult T-cell leukemia lymphoma (ATLL); a/d T-NHL hepatosplenic lymphoma; peripheral/post-thymic T cell lymphoma of the pleomorphic or immunoblastic subtype; angio immunoblastic T-cell lymphoma; angiocentric (nasal) T-cell lymphoma; anaplastic (Ki 1+) large cell lymphoma; intestinal T-cell lymphoma; T-lymphoblastic leukemia; or lymphoma/leukemia (T-Lbly/T-ALL).

47. The method of claim 42, wherein said patient is suffering from a proliferative disorder selected from hyperplasias, fibrosis, angiogenesis, psoriasis, atherosclerosis, stenosis or restenosis following angioplasty, and other diseases characterized by smooth

muscle proliferation in blood vessels.

48. The method of claim 42, wherein said patient is suffering from an infectious disease caused by a virus selected from hepatitis type A, hepatitis type B, hepatitis type C, influenza, varicella, adenovirus, herpes simplex type I (HSV-1), herpes simplex type 2 (HSV-2), rinderpest, rhinovirus, echovirus, rotavirus, respiratory syncytial virus, papilloma virus, papilloma virus, cytomegalovirus, echinovirus, arbovirus, huntavirus, coxsackie virus, mumps virus, measles virus, rubella virus, polio virus or human immunodeficiency virus type I or type 2 (HIV-1, HIV-2).

49. The method of claim 42, wherein said patient is suffering from an infectious disease caused by a bacteria, protozoa or parasite selected from Staphylococcus, S. pyogenes, Enterococcl, Bacillus anthracis, Lactobacillus, Listeria, Corynebacterium diphtheriae, G. vaginalis; Nocardia; Streptomyces; Thermoactinomyces vulgaris; Treponerna; Camplyobacter, Raeruginosa; Legionella; N.gonorrhoeae; N.meningitides; F. meningosepticum; F. odoraturn; Brucella; B. pertussis; B. bronchiseptica; E. coli; Klebsiella; Enterobacter; S. marcescens; S. liquefaciens; Edwardsiella; P. mirabilis; P. vulgaris; Streptobacillus; R. fickettsfi; C. psittaci; C. trachornatis; M. tuberculosis, M. intracellulare, M. folluiturn, M. laprae, M. avium, M. bovis, M. africanum, M. kansasii, M. intracellulare; M. lepraernurium; Nocardia, other Streptococcus, other Bacillus, other Gardnerella, other Pseudomonas, other Neisseria, other Flavobacterium, other Bordetella, other Escherichia, other Serratia, other Proteus, other Rickettsiaceae, other Chlamydia, other Mycobacterium, leishmania, kokzidioa, trypanosome, chlamydia or rickettsia.

50. The method according to claim 42, comprising the additional step of administering to said patient an appropriate additional therapeutic agent selected from an immunomodulatory agent, a hormonal agent, a chemotherapeutic agent, an anti-angiogenic agent, an apoptotic agent, a second antibody that binds to and inhibits an inhibitory KIR receptor, an anti -infective agent, a targeting agent or an adjunct compound wherein said additional therapeutic agent is administered to said patient as a

single dosage form together with said antibody, or as separate dosage form.

51. The antibody of claim 1, wherein said antibody is conjugated or covalently bound to a toxin, a detectable moiety, or a solid support.

52. A method of detecting the presence of NK cells bearing an inhibitory KIR on their cell surface in a biological sample or a living organism, said method comprising the steps of:

a) contacting said biological sample or living organism with an antibody of claim 51, wherein said antibody is conjugated or covalently bound to a detectable moiety; and

b) detecting the presence of said antibody in said biological sample or living organism.

53. A method of purifying from a sample NK cells bearing an inhibitory KIR on their cell surface comprising the steps of:

a) contacting said sample with an antibody of claim 51 under conditions that allow said NK cells bearing an inhibitory KIR on their cell surface to bind to said antibody, wherein said antibody is conjugated or covalently bound to a solid support; and

b) eluting said bound NK cells from said antibody conjugated or covalently bound to a solid support.

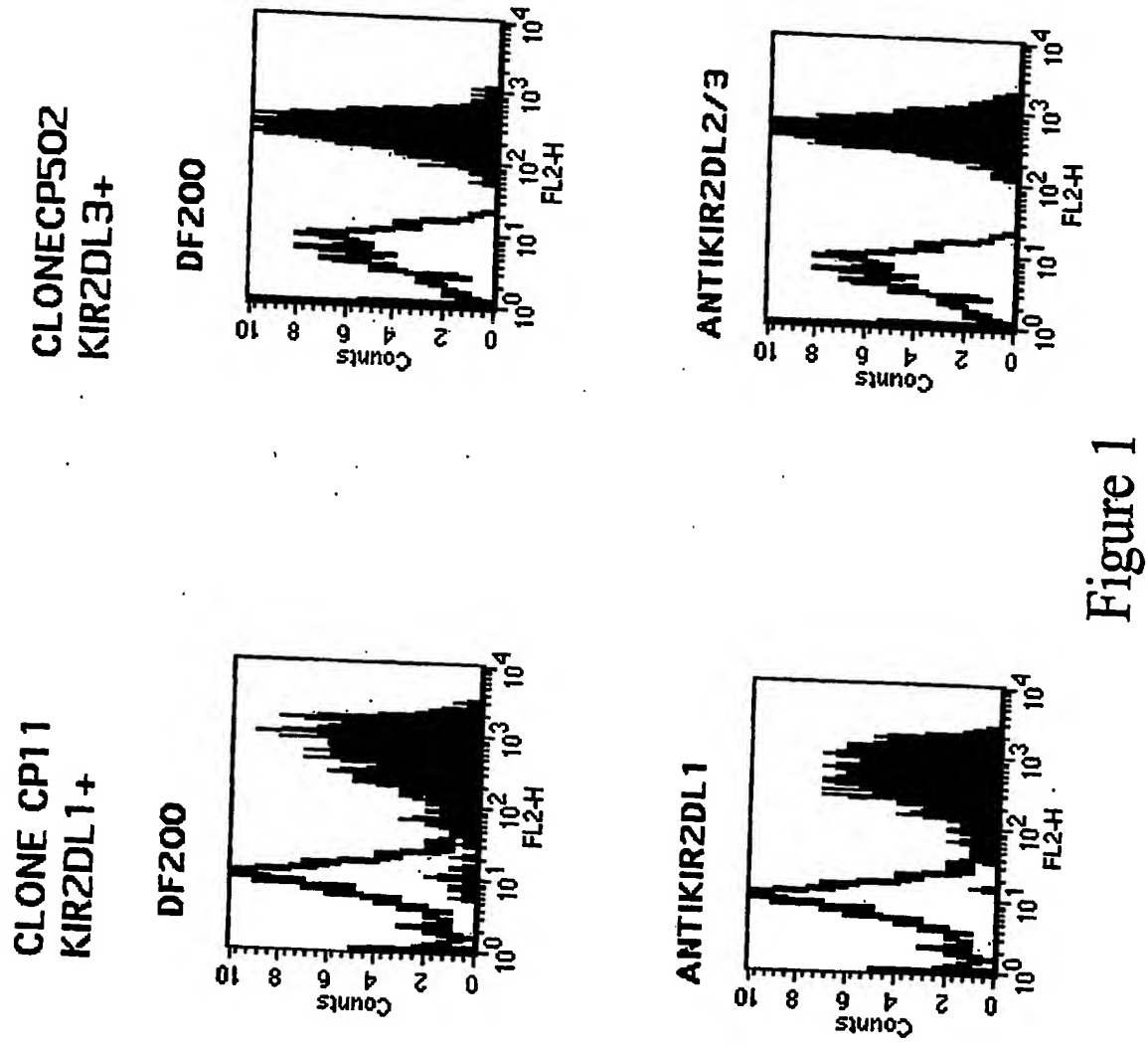
54. A composition comprising an antibody that binds a common determinant present on at least two different human inhibitory KIR receptor gene products, wherein said antibody is capable of neutralizing KIR-mediated inhibition of NK cell cytotoxicity on NK cells expressing at least one of said two different human inhibitory KIR receptors, wherein said antibody is incorporated into a liposome.

55. The composition of claim 54, wherein an additional substance selected from a nucleic acid molecule for the delivery of genes for gene therapy; a nucleic acid molecule for the delivery of antisense RNA, RNAi or siRNA for suppressing a gene in an NK cell;

or a toxin or a drug for the targeted killing of NK cells is additionally incorporated into said liposome.

Abstract

[00135] The present invention relates to novel compositions and methods for regulating an immune response in a subject. More particularly, the invention relates to specific antibodies that regulate the activity of NK cells and allow a potentiation of NK cell cytotoxicity in mammalian subjects. The invention also relates to fragments and derivatives of such antibodies, as well as pharmaceutical compositions comprising the same and their uses, particularly in therapy, to increase NK cell activity or cytotoxicity in subjects.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KIR2DL3+
DF200
ANTIKIR2DL2/3
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DF200
ANTIKIR2DL1
Counts
FL2-H

Figure 1

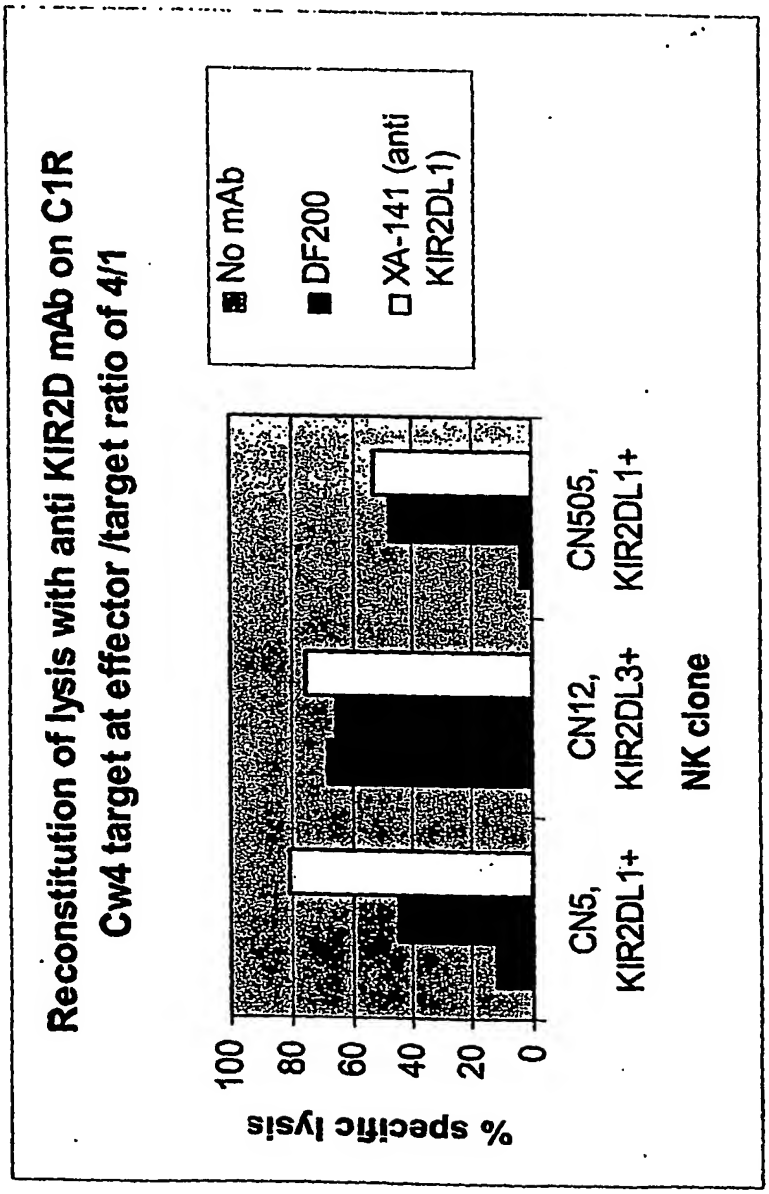

Figure 2